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Figure 1: Biosynthetic scheme of carotenoids in tomato flowers

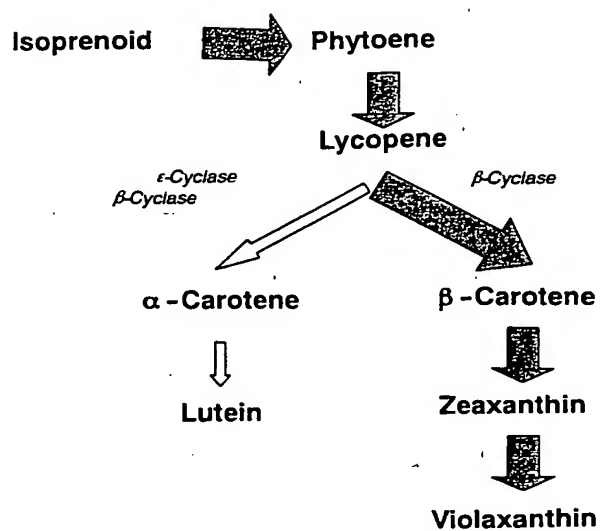
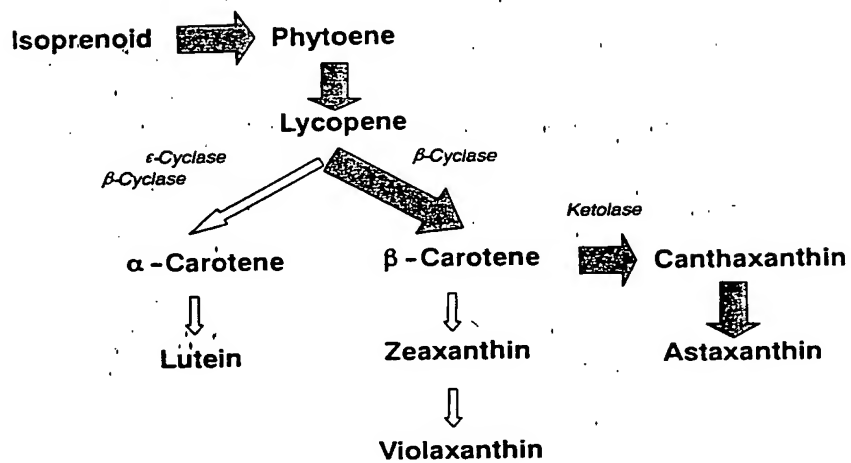


Figure 2: Biosynthetic scheme of Astaxanthin in genetically modified flowers



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Figure 3: Nucleotide sequence alignment

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KETO2.seq  ATGCAGCTAGCAGCGACAGTAATGTTGGAGCAGCTTACCGGAAGCGCTGAGGCACCTCAAGGAGAAAGGAGAGGTTCCAGCCAGCTCTGACGTGTTCC 100
X86782.seq  ATGCAGCTAGCAGCGACAGTAATGTTGGAGCAGCTTACCGGAAGCGCTGAGGCACCTCAAGGAGAAAGGAGAGGTTCCAGCCAGCTCTGACGTGTTCC 100

KETO2.seq  GTACATGCGCGACCCAGTACTCGCTTCGGTCAGAGAGTCAGAGCGCGCGCGCGCGCGGACTGAAGAATGCTTACAAGCCAGCAGCTTCCGACACAAAGGG 200
X86782.seq  GTACATGCGCGACCCAGTACTCGCTTCGGTCAGAGAGTCAGAGCGCGCGCGCGCGGACTGAAGAATGCTTACAAGCCAGCAGCTTCCGACACAAAGGG 200

KETO2.seq  CATCACAATGCGCGTACGTGTCATCGGCTCCTGCGCGCGCAGTGTTCCTCCAGCCCATTTTTCAAATCAAGCTTCCGAGCTCCTTGGACCACTGCCTGG 300
X86782.seq  CATCACAATGCGCGTACGTGTCATCGGCTCCTGCGCGCGCAGTGTTCCTCCAGCCCATTTTTCAAATCAAGCTTCCGAGCTCCTTGGACCACTGCCTGG 300

KETO2.seq  CTGCGCGGTGTCAGATGCCACAGCTCAGCTGGTTAGCGCGCAGCAGCAGCTGCTGCACATGCTGCTAGTATTCTTTGTCTGGAGTTCTGTACACAGGCC 400
X86782.seq  CTGCGCGGTGTCAGATGCCACAGCTCAGCTGGTTAGCGCGCAGCAGCAGCTGCTGCACATGCTGCTAGTATTCTTTGTCTGGAGTTCTGTACACAGGCC 400

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X86782.seq  TTTTATACACAGCCATGATGCTATGCATGCCACCATGCCCATGAGAAACAGCCAGCTTAATGACTTCTTGGGCAGAGTATGCATCTCCTTGTACGCCCTG 500

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X86782.seq  GTTTGATTACAACATGCTGCACCGCAAGCATTTGGAGCACCACAACACACTGGGAGGTGGGCAAGGAGCCCTGACTTCCACAGCGGAAAGCCCTGCCATT 600

KETO2.seq  GTGCGCGTGGTTTCCAGCTTCATGTCCAGCTACATGTGGATGTGGCAGTTTGGCGCGCTGGCATGGTGGAGCGTGGTCAATGCACTCCTCGGTGGCCAA 700
X86782.seq  GTGCGCGTGGTTTCCAGCTTCATGTCCAGCTACATGTGGATGTGGCAGTTTGGCGCGCTGGCATGGTGGAGCGTGGTCAATGCACTCCTCGGTGGCCAA 700

KETO2.seq  TGGGAAAGCTGCTGGTGTTCATGGCGCGCGCGCGCGCATGCTGTGCGGCTTCCGCTTGTCTACTTTGGCAGTACATGCGCGCACAGCCCTGAGCCCTGGCGC 800
X86782.seq  TGGGAAAGCTGCTGGTGTTCATGGCGCGCGCGCGCGCATGCTGTGCGGCTTCCGCTTGTCTACTTTGGCAGTACATGCGCGCACAGCCCTGAGCCCTGGCGC 800

KETO2.seq  CCGGTCAGGCTCTTCAACAGCGGTCATGAACCTGGTGGAGTGGCGCACTAGCCAGCGGTGGAGCTGGTCAGCTTCTGAGCTCTACCACTTGGAGCTG 900
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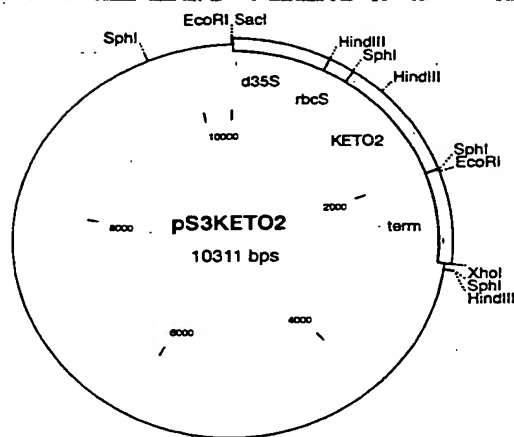
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Figure 4: Protein sequence alignment

KETO2.pro	MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATOYSLPSEESDAA	50
X86782.pro	MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATOYSLPSEESDAA	50
KETO2.pro	RPGLKNAYKPPPSDTKGITMALAVIGSWAAVFLHAIFOIKLPTSLDQLHW	100
X86782.pro	RPGLKNAYKPPPSDTKGITMALRVIGSWAAVFLHAIFOIKLPTSLDQLHW	100
KETO2.pro	LPVSDATAQLVSGSSSLLHIVVVFFVLEFLYTGLFITTHDAMHGTTIAMRN	150
X86782.pro	LPVSDATAQLVSGTSSLLDIVVVFFVLEFLYTGLFITTHDAMHGTTIAMRN	150
KETO2.pro	RQLNDFLGRVCI SLYAWFDYNMLHRKHWEHHNHTGEVCKDPDFHRGNPGI	200
X86782.pro	RQLNDFLGRVCI SLYAWFDYNMLHRKHWEHHNHTGEVCKDPDFHRGNPGI	200
KETO2.pro	VPWFASFMS SYMS MWQFARLAWWT VVMQLLGAPMANLLVFMAAAPILSAF	250
X86782.pro	VPWFASFMS SYMS MWQFARLAWWT VVMQLLGAPMANLLVFMAAAPILSAF	250
KETO2.pro	RLFYFGTYMPHKPEPGAASGSSPAVMNWWKSRTSQASDLVSFLTCTYHFDL	300
X86782.pro	RLFYFGTYMPHKPEPGAASGSSPAVMNWWKSRTSQASDLVSFLTCTYHFDL	300
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X86782.pro	HWEHHRWPFAPWWELPNCRRLSGRGLVPA	329

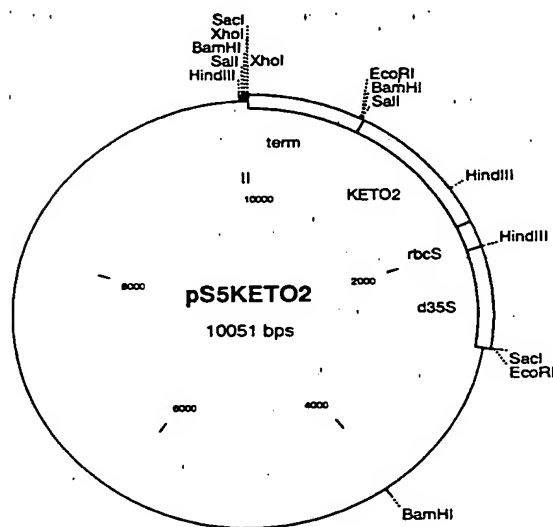
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Figure 5A: Construct for overexpressing the ketolase (β -C-4-oxygenase) protein from *H. pluvialis* with *rbcS* transit peptide from pea under the control of the d35S-promoter (tomato transformation construct)



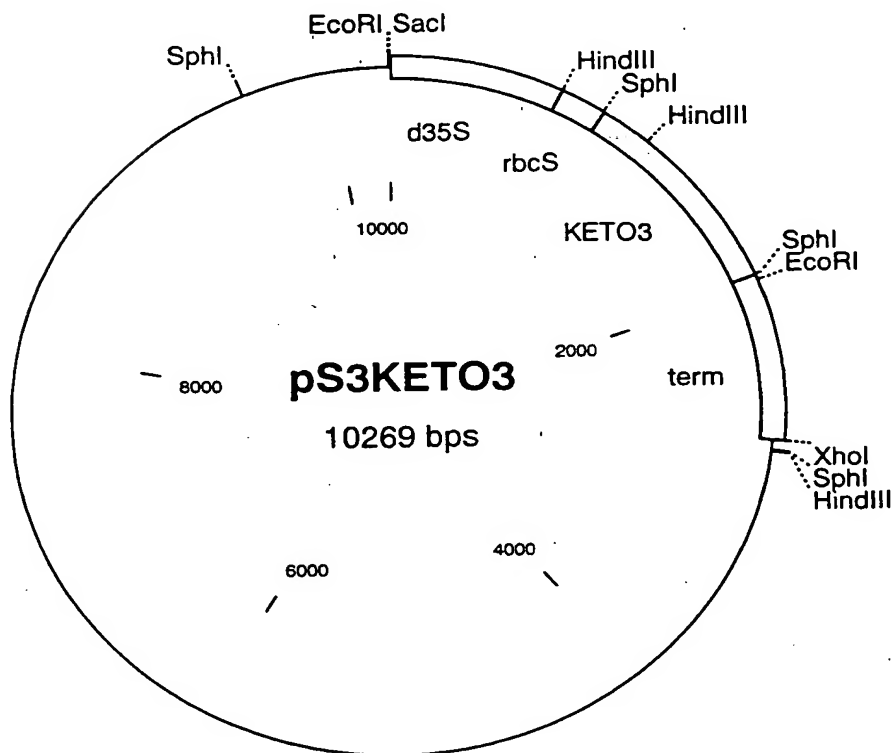
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Figure 5B: Construct for overexpressing the ketolase (β -C-4-oxygenase) protein from *H. pluvialis* with *rbcS* transit peptide from pea under the control of the d35S-promoter (Tagetes transformation construct)



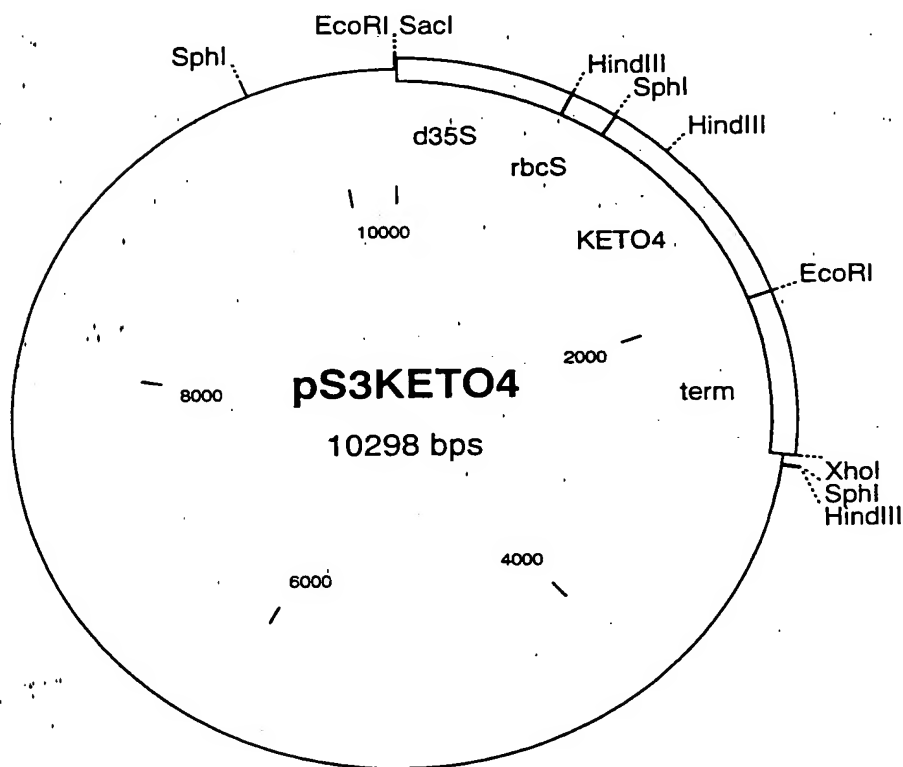
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Figure 6: Construct for overexpressing the N-terminally truncated ketolase (β -C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea under the control of the d35S promoter.



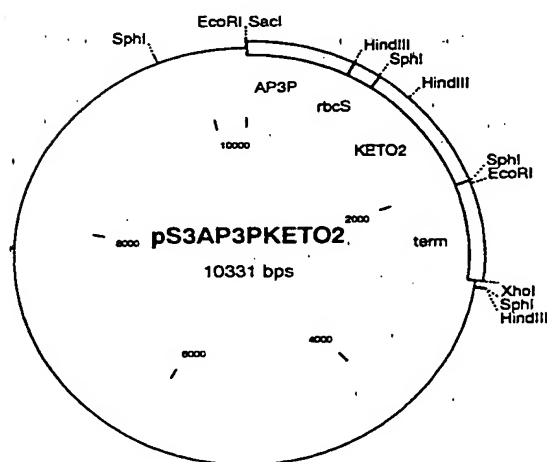
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Figure 7: Construct for overexpressing the ketolase (β -C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea and C-terminal myc tag under the control of the d35S promoter.



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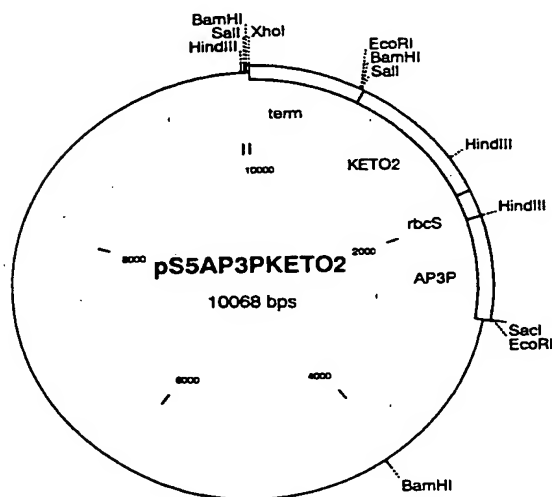
Figure 8A: Construct pS3AP3PKETO2 for overexpressing the ketolase (β -C-4-oxygenase) proteins from *H. pluvialis* with *rbcS* transit peptide from pea under the control of the AP3P promoter (tomato transformation construct).



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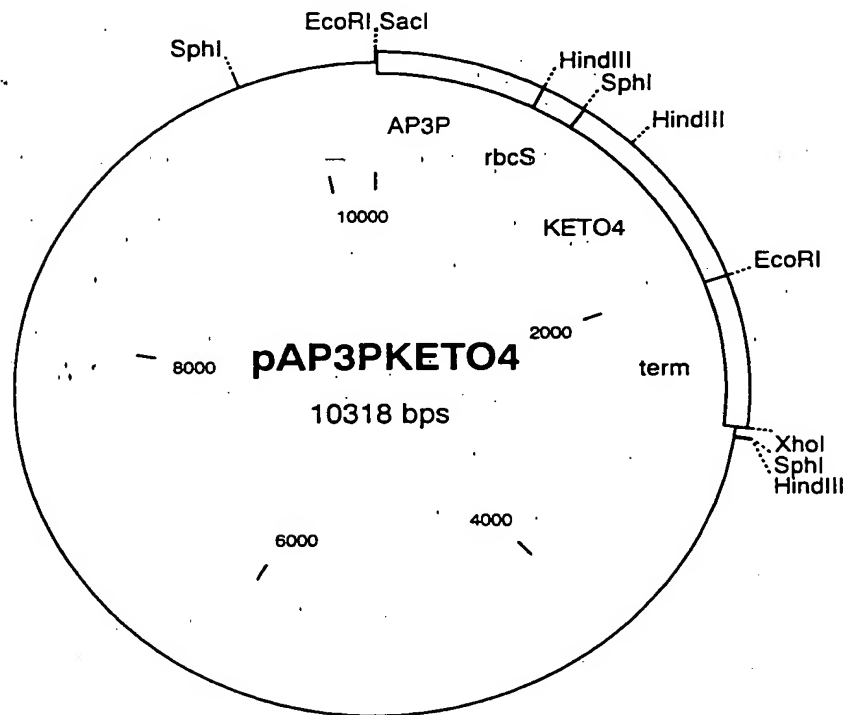
Figure 8B:

Construct pS3AP3PKETO2 for overexpressing the ketolase (β -C-4-oxygenase) proteins from *H. pluvialis* with *rbcS* transit peptide from pea under the control of the AP3P promoter (Tagetes transformation construct).



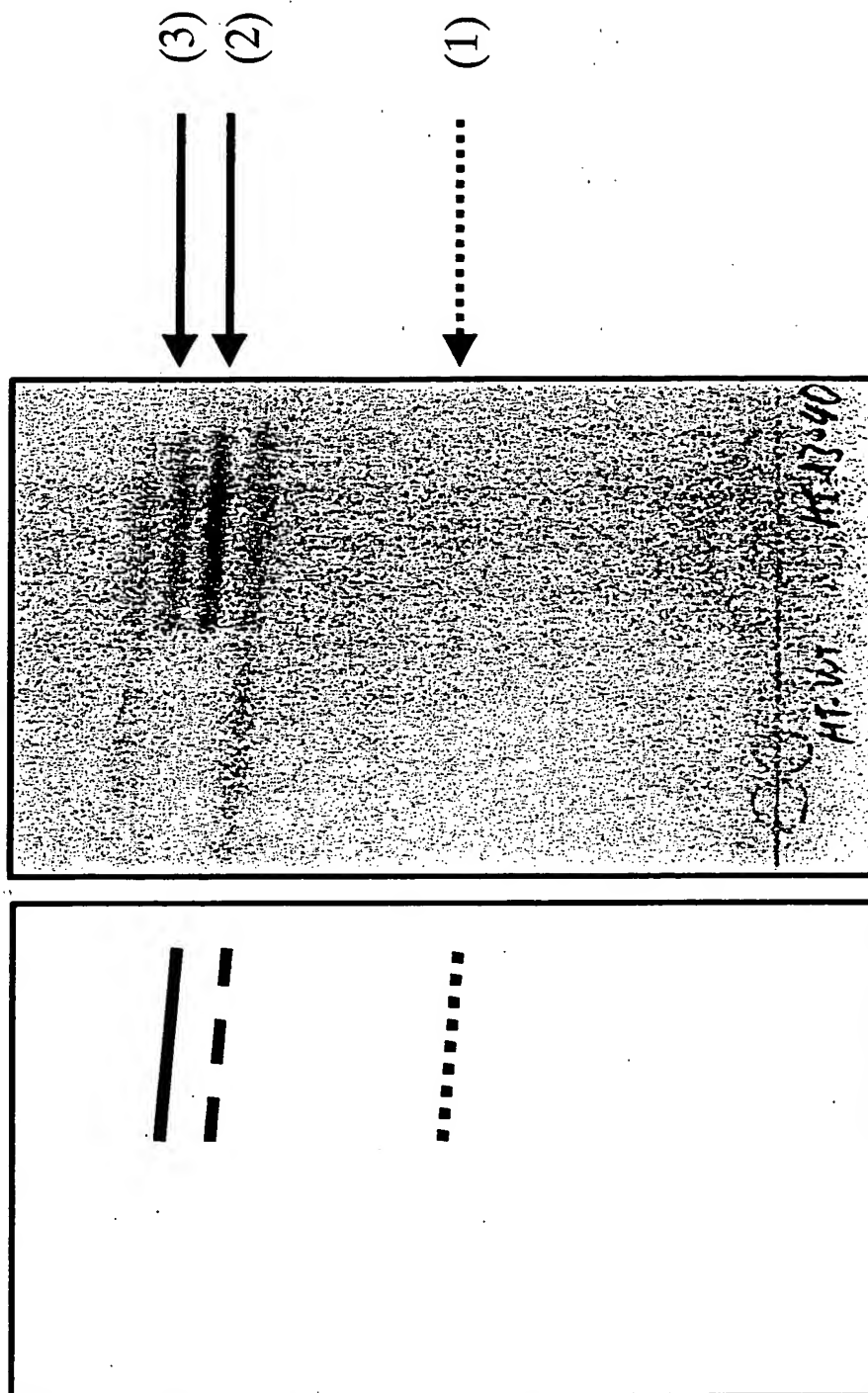
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Figure 9: Construct for overexpressing the ketolase (β -C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea and C-terminal myc tag under the control of the AP3P promoter.



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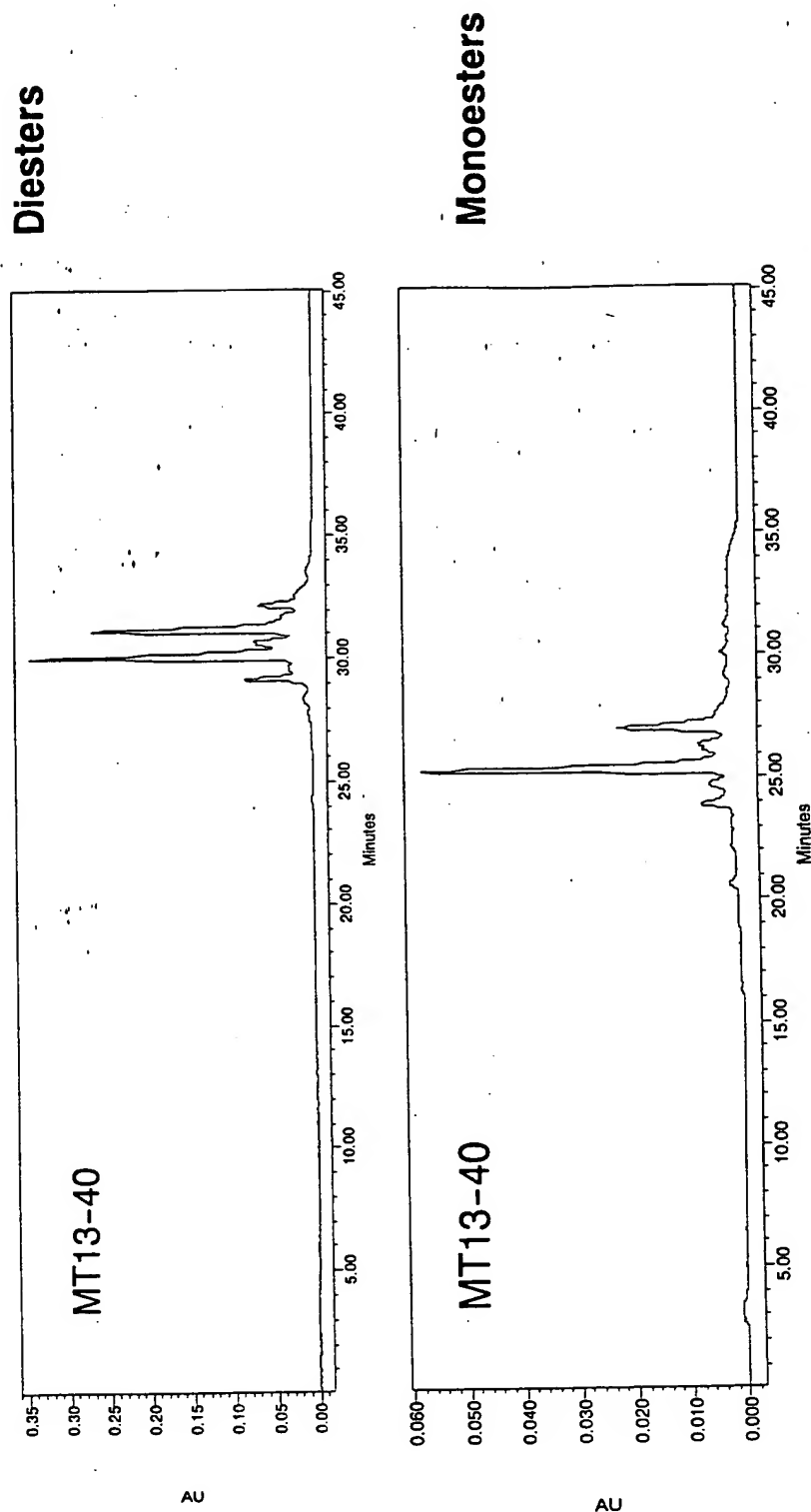
Figure 9a: Ester separation by means of thin-layer chromatography



Left: schematic representation; right: photograph of the thin-layer plate. (3) and (2) indicate ketocarotenoid diesters, (1) ketocarotenoid monoesters

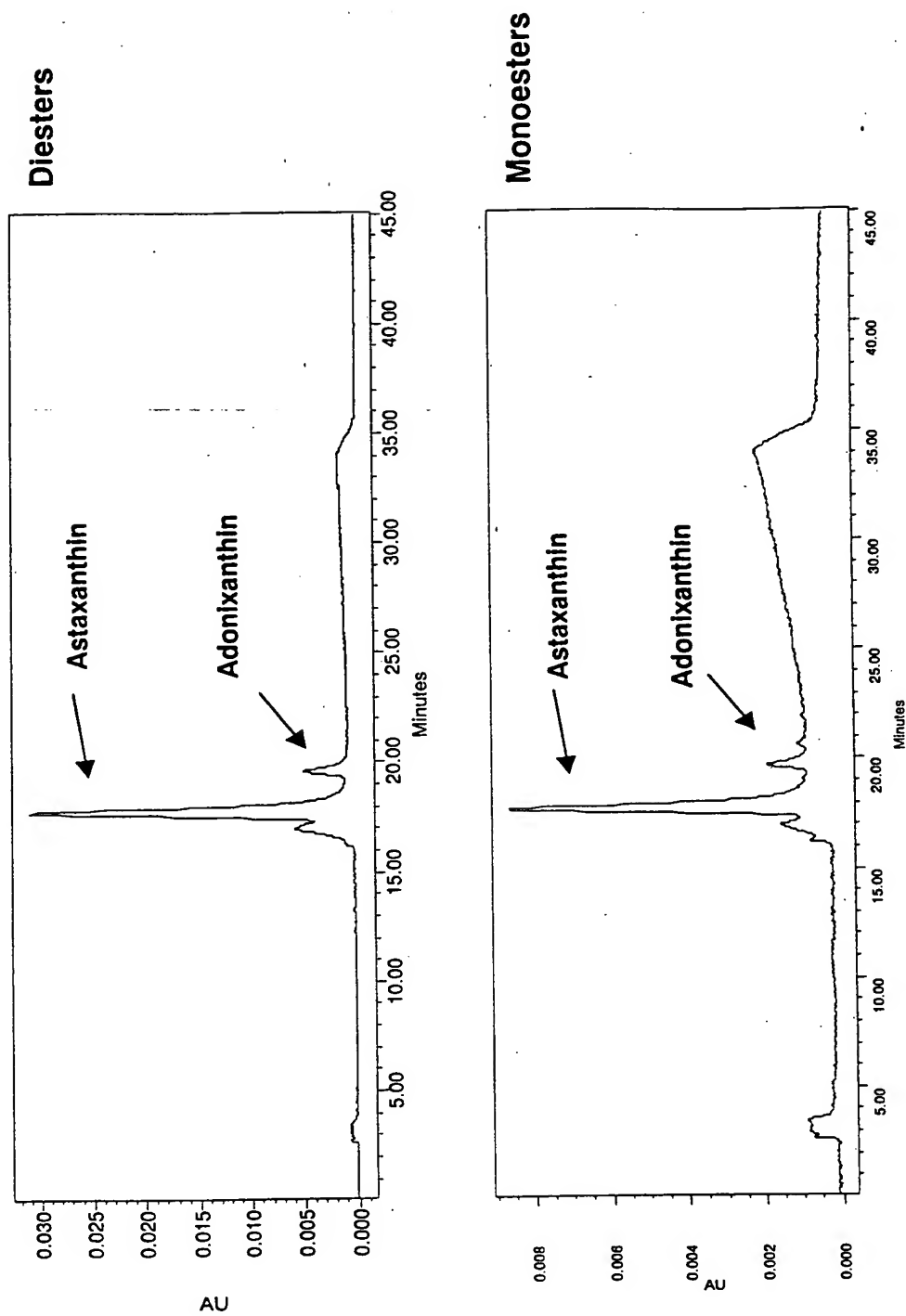
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Fig. 10



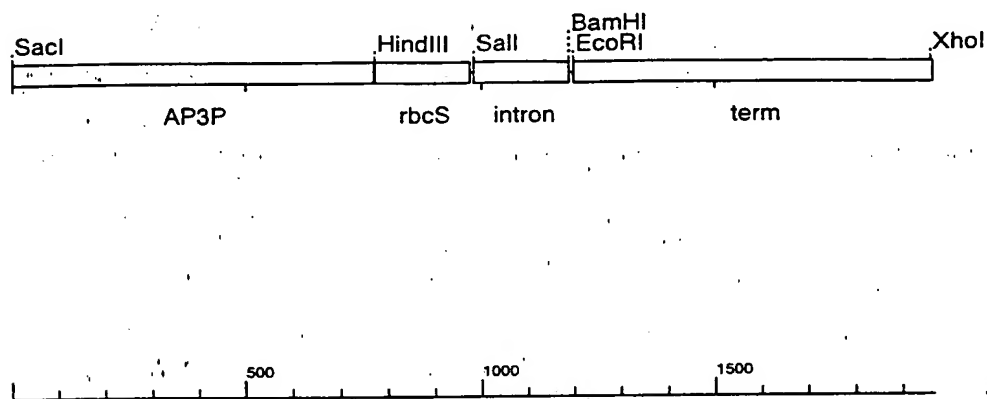
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Fig. 11



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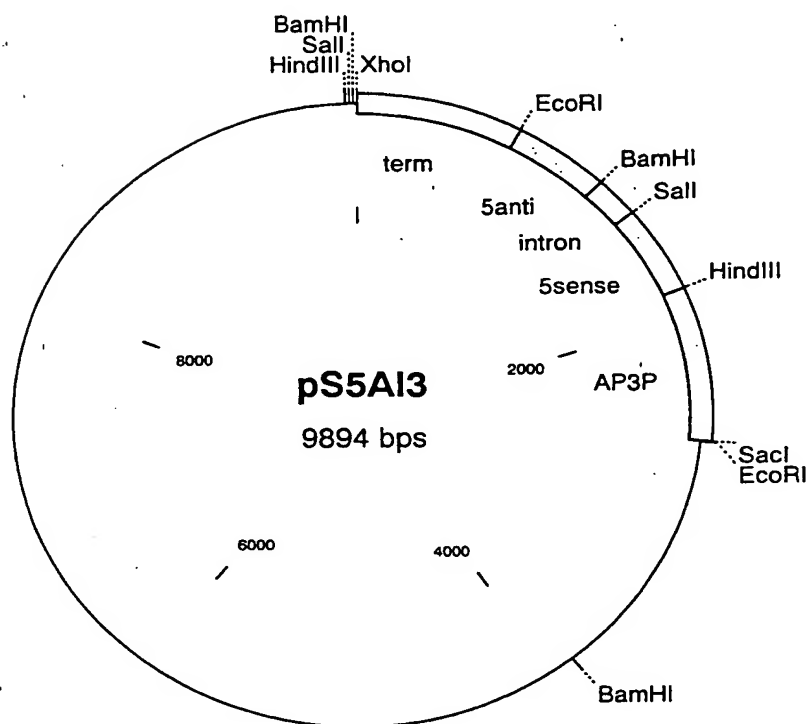
Figure 12: Cloning cassette for the preparation of inverted-repeat expression cassettes for the flower-specific expression of epsilon-cyclase dsRNAs in *Tagetes erecta*



pJA11 (1966 bps)

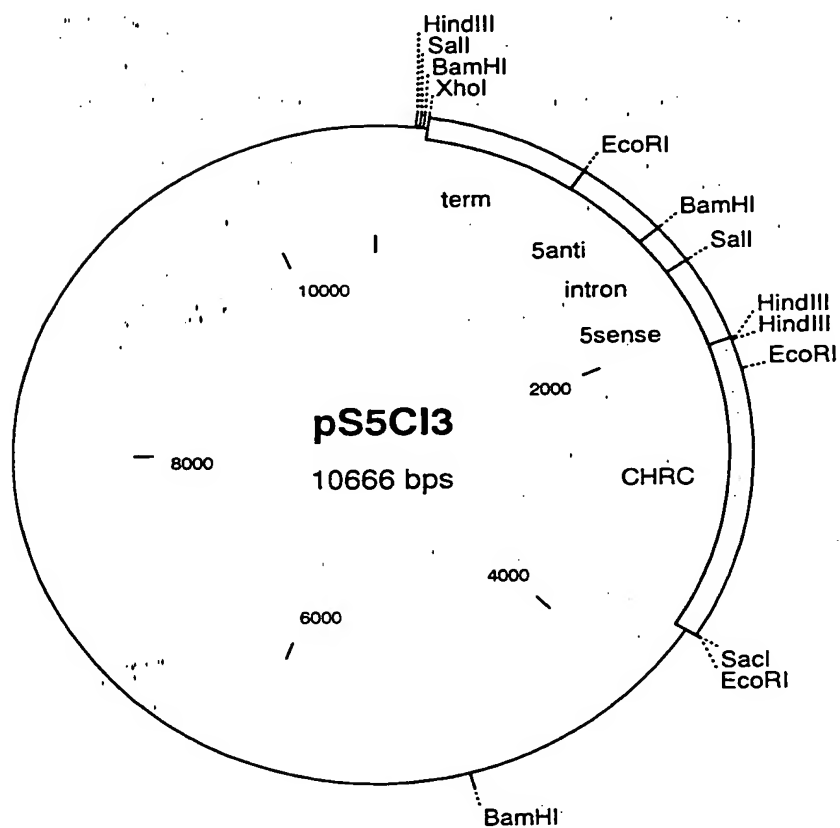
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Figure 13: Expression vector for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter



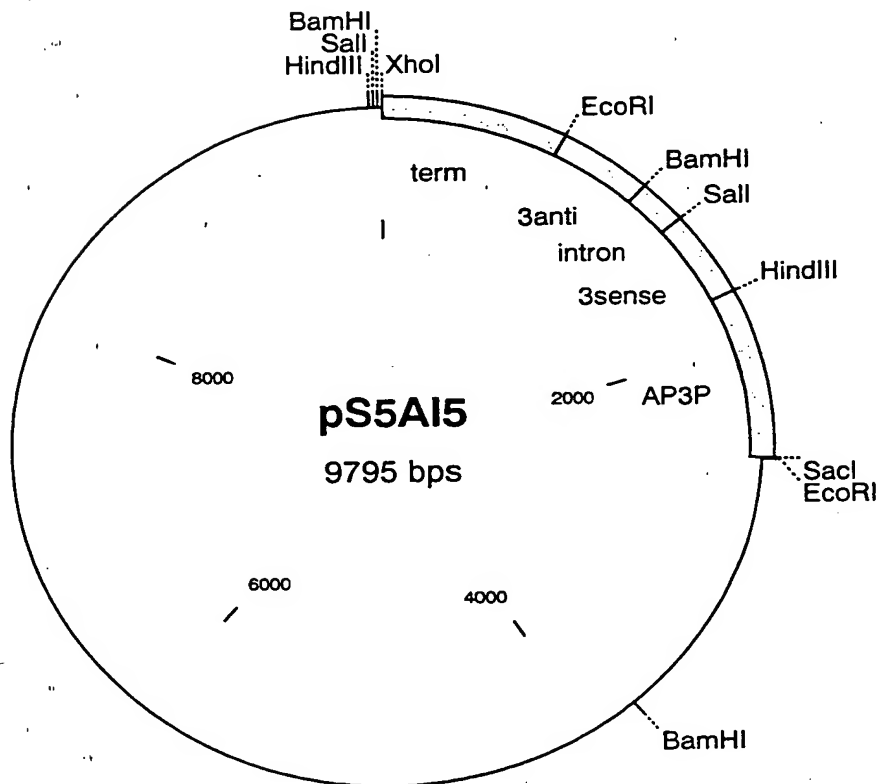
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Figure 14: Expression vector for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the CHRC promoter



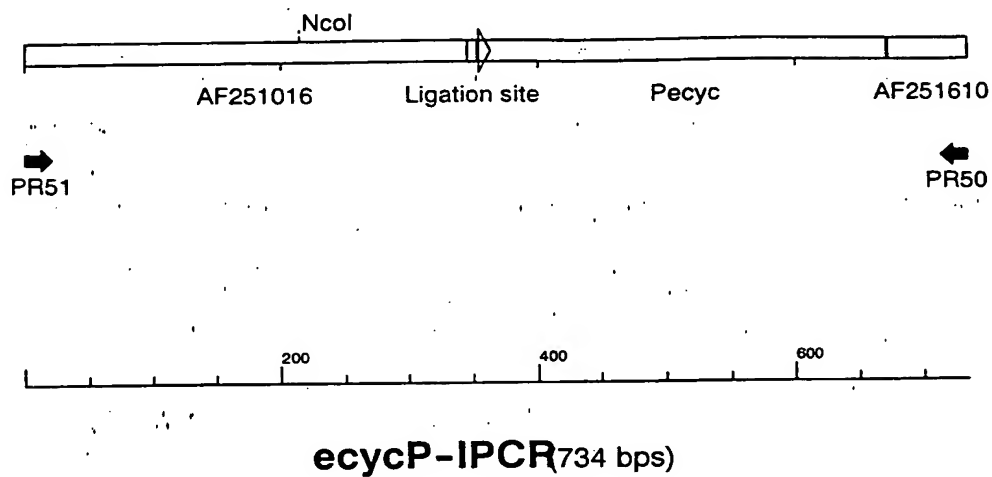
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Figure 15: Expression vector for the flower-specific production of dsRNA transcripts comprising 3'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter



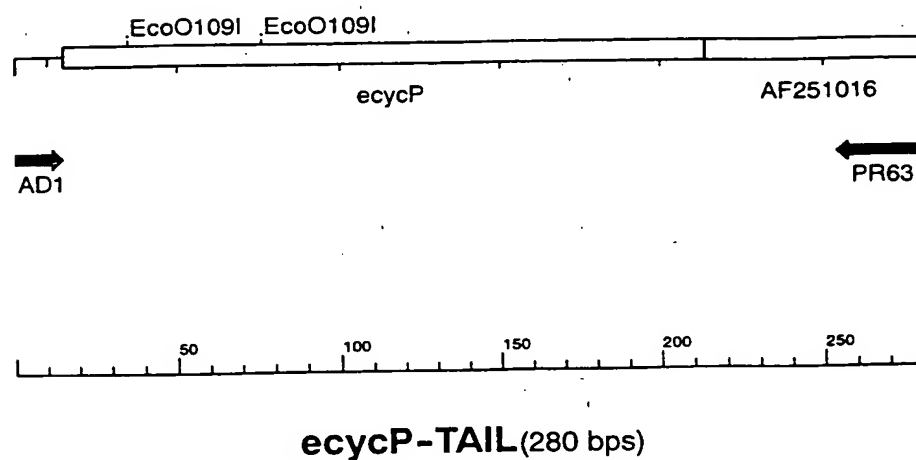
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Figure 16: Inverse PCR amplificate comprising the 312 bp fragment of the epsilon-cyclase promoter



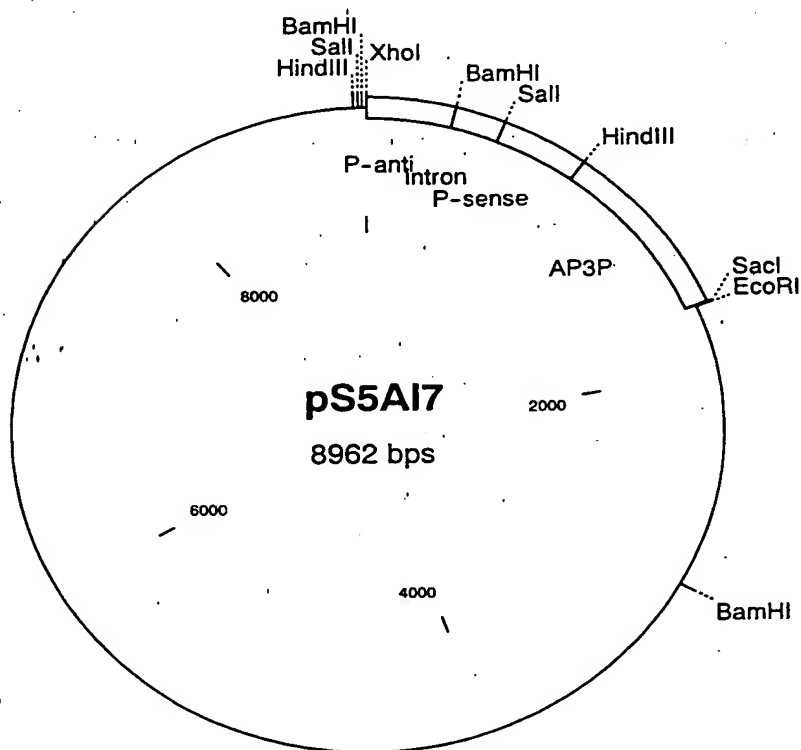
20/47

Figure 17: TAIL PCR amplificate comprising the 199 bp fragment of the epsilon-cyclase promoter



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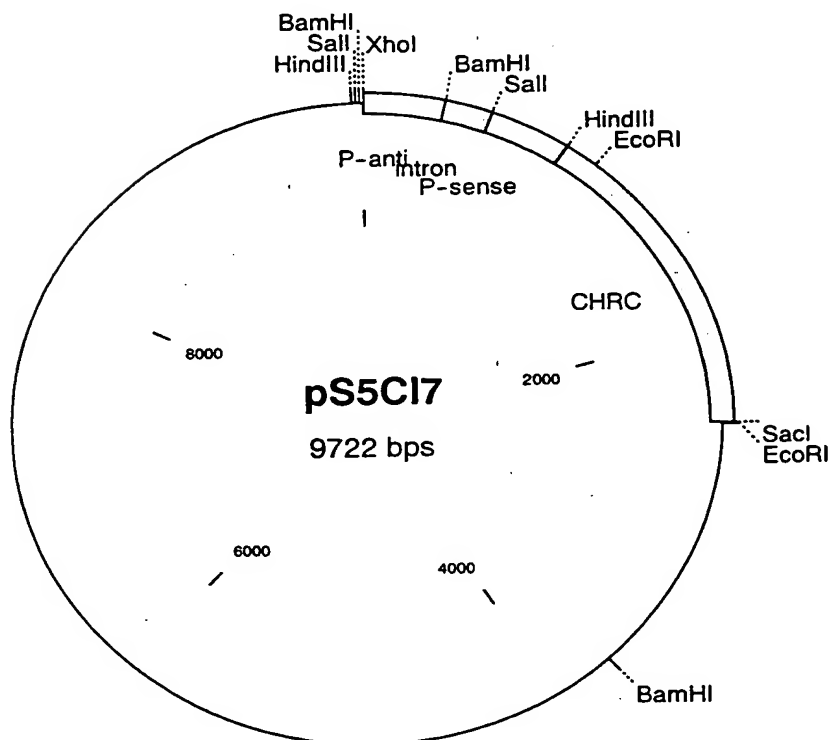
Figure 18: Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the AP3P promoter



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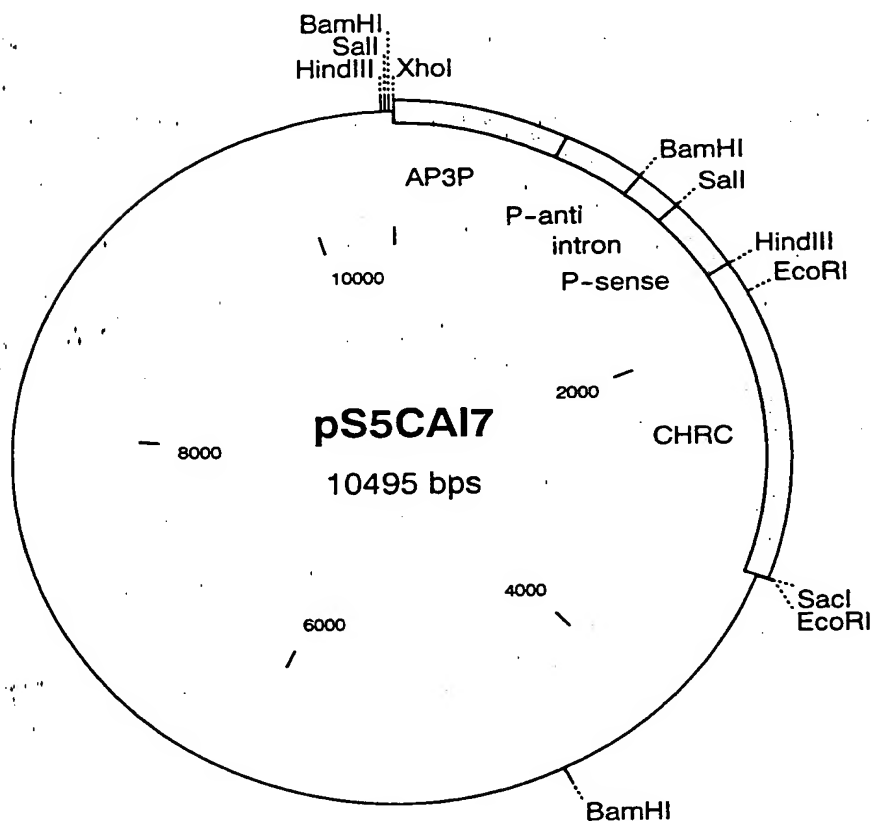
Figure 19:

Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the CHRC promoter



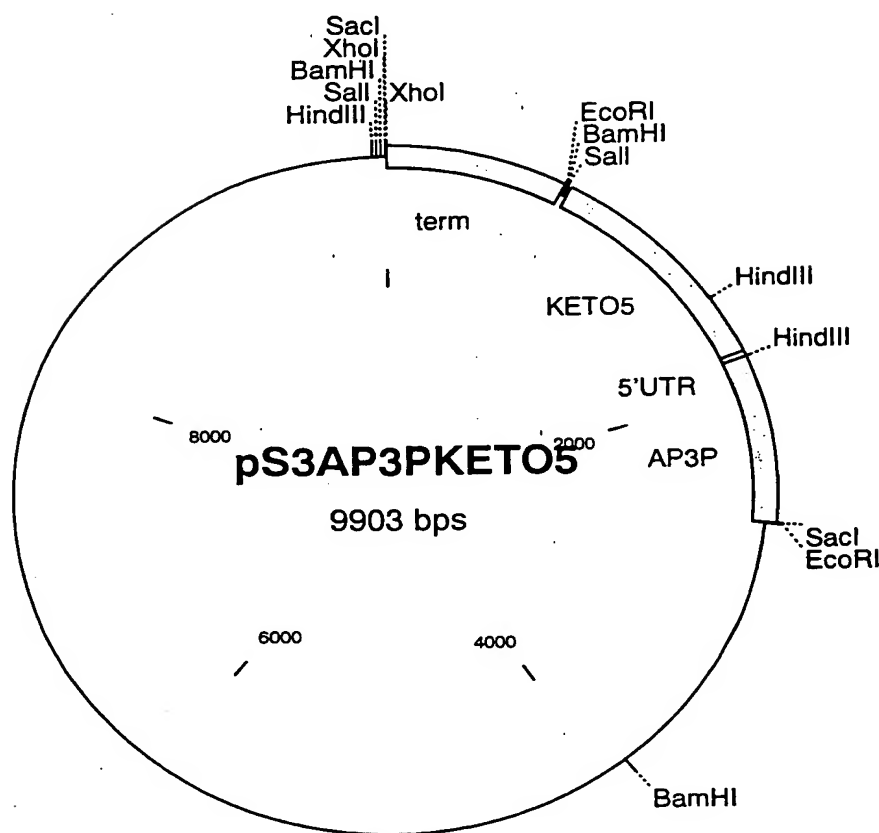
23/47

Figure 20: Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the AP3P promoter and of the CHRC promoter



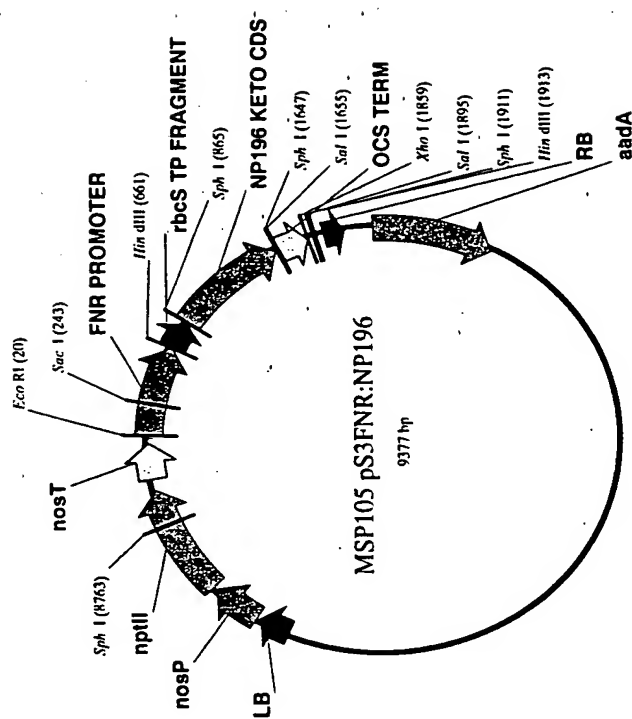
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Figure 21: Construct for the flower-specific overexpression of the ketolase (β -C-4-oxygenase) protein from *H. pluvialis* without heterologous transit peptide.



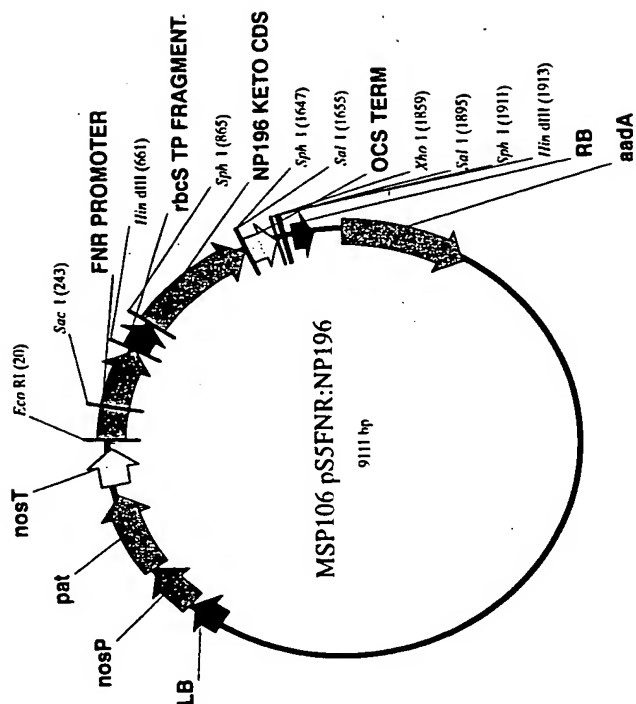
25/47

Figure 22: pSUN3 construct for overexpressing the β -C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter



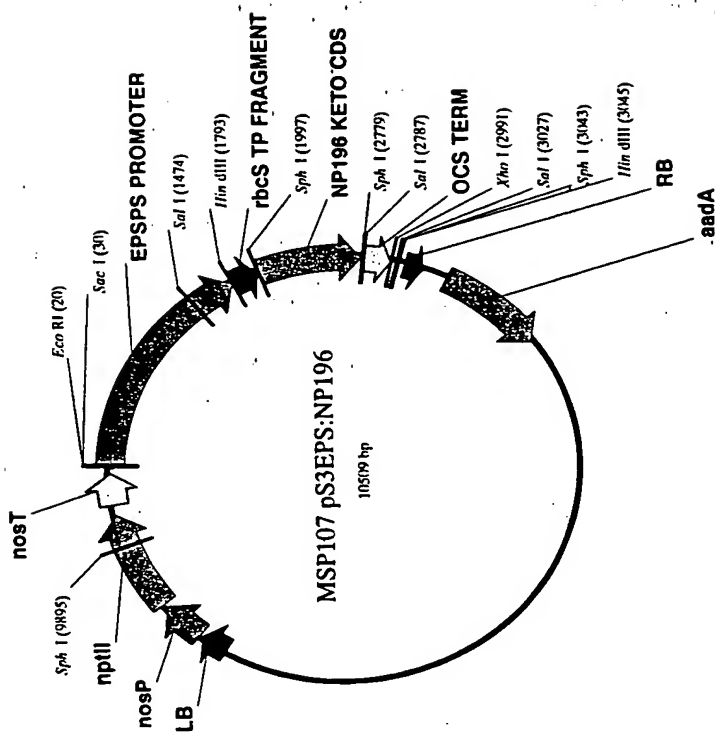
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Figure 23: pSUN5 construct for overexpressing the β -C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter



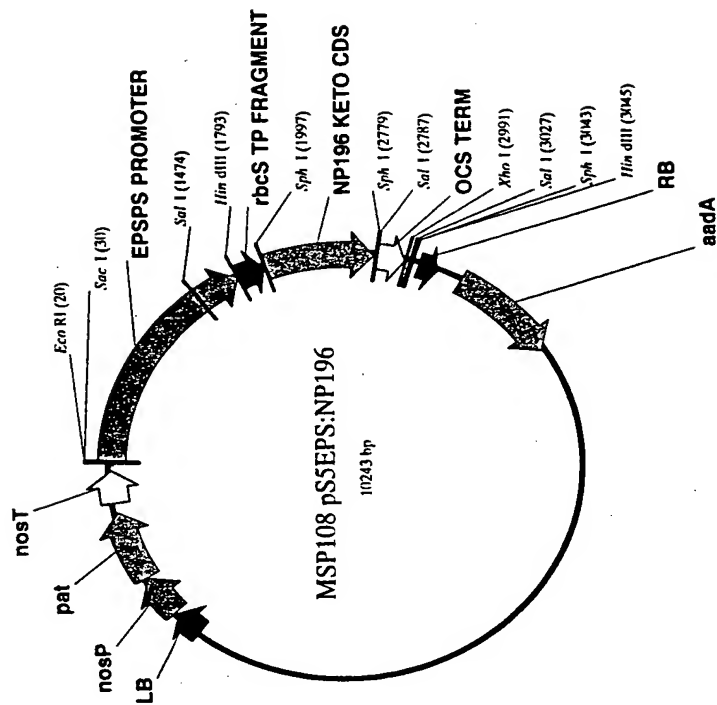
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Figure 24: pSUN3 construct for overexpressing the β -C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter



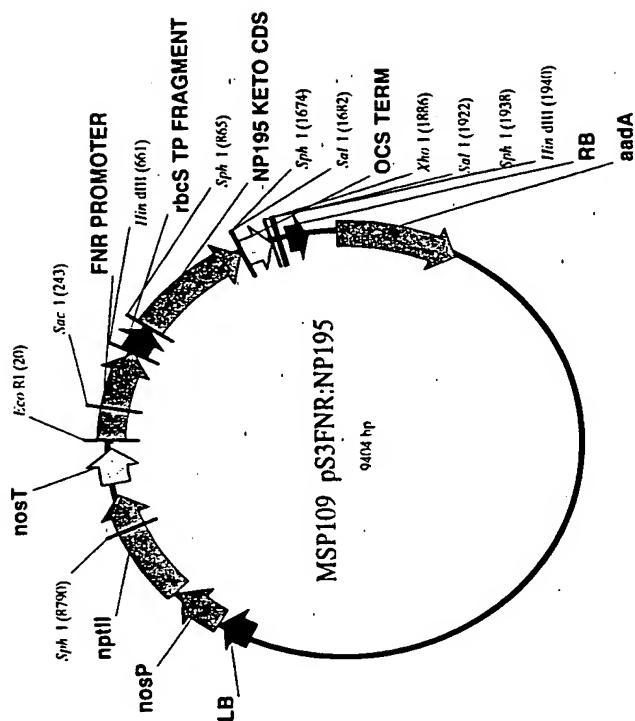
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Figure 25: pSUN5 construct for overexpressing the β -C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter



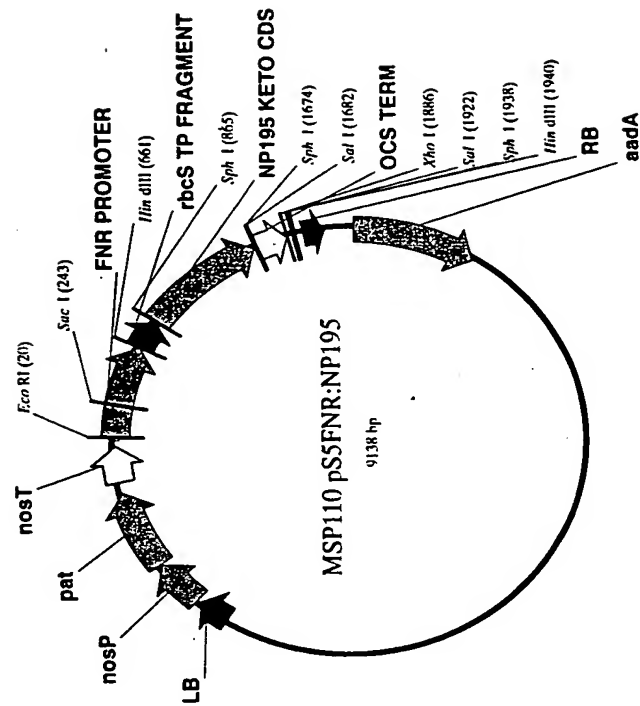
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Figure 26: pSUN3 construct for overexpressing the β -C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter



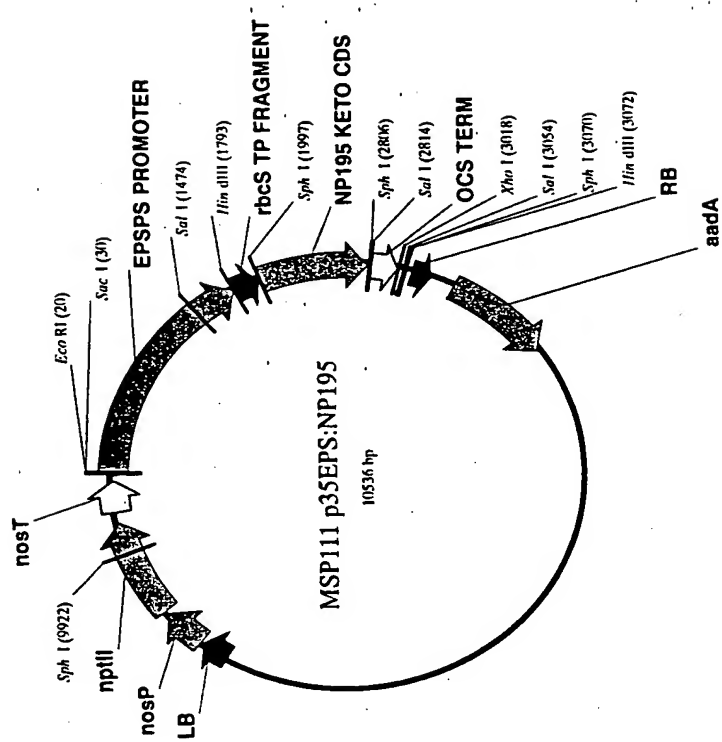
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Figure 27: psUN5 construct for overexpressing the β -C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter



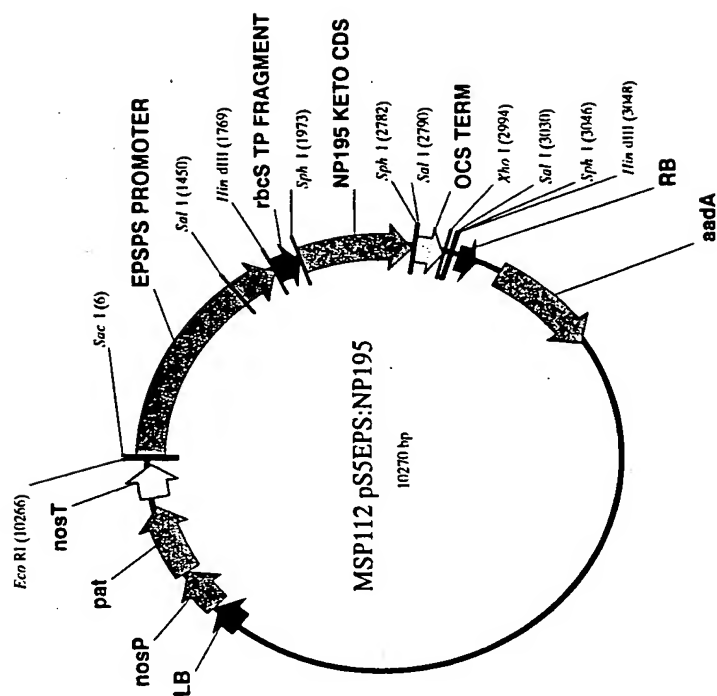
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Figure 28: pSUN3 construct for overexpressing the β -C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter



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Figure 29: pSUN5 construct for overexpressing the β -C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with *rbcS* transit peptide from pea under the control of the EPSPS promoter



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Figure 30: pSUN3 construct for overexpressing the β -C-4-oxygenase protein from *Nodularia spumigena* NSOR10 with rbcS transit peptide from pea under the control of the FNR promoter

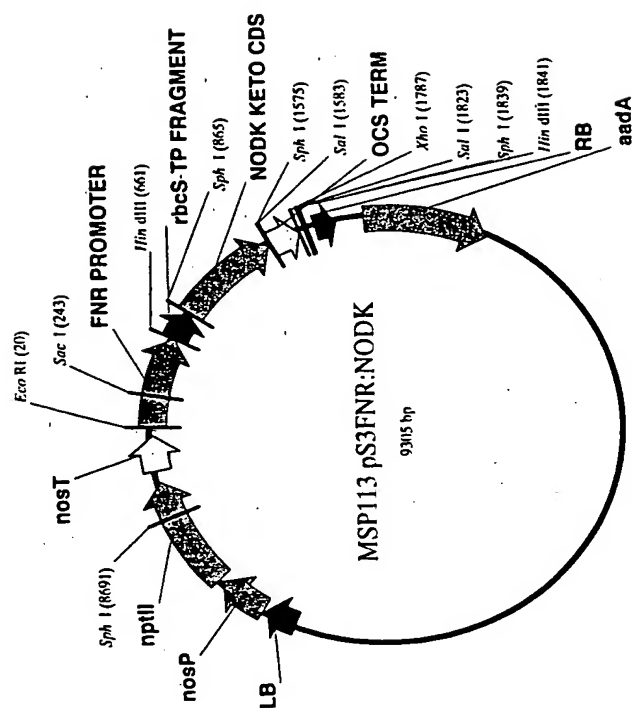
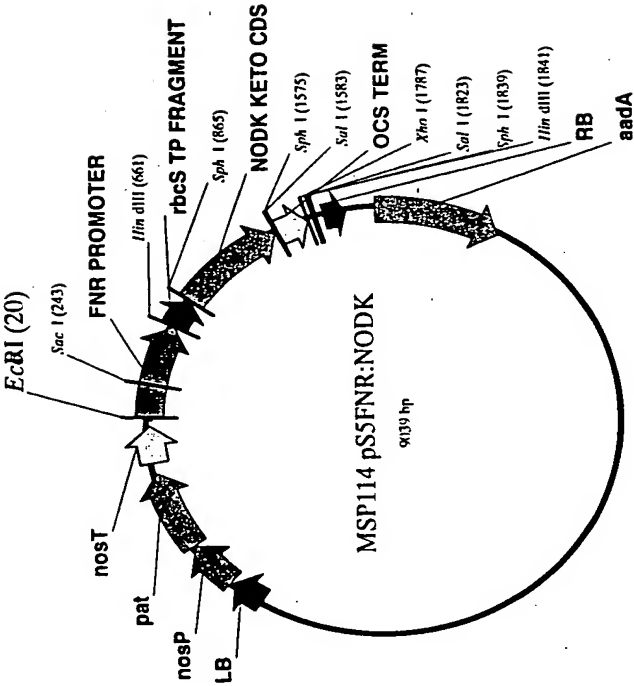


Figure 31: pSUN5 construct for overexpressing the β -C-4-oxygenase protein from *Nodularia spumigena* NSOR10 with rbcS transit peptide from pea under the control of the FNR promoter



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Figure 32: psUN3 construct for overexpressing the β -C-4-oxygenase protein from *Nodularia spumigena* NSOR10 with rbcS transit peptide from pea under the control of the EPSPS promoter

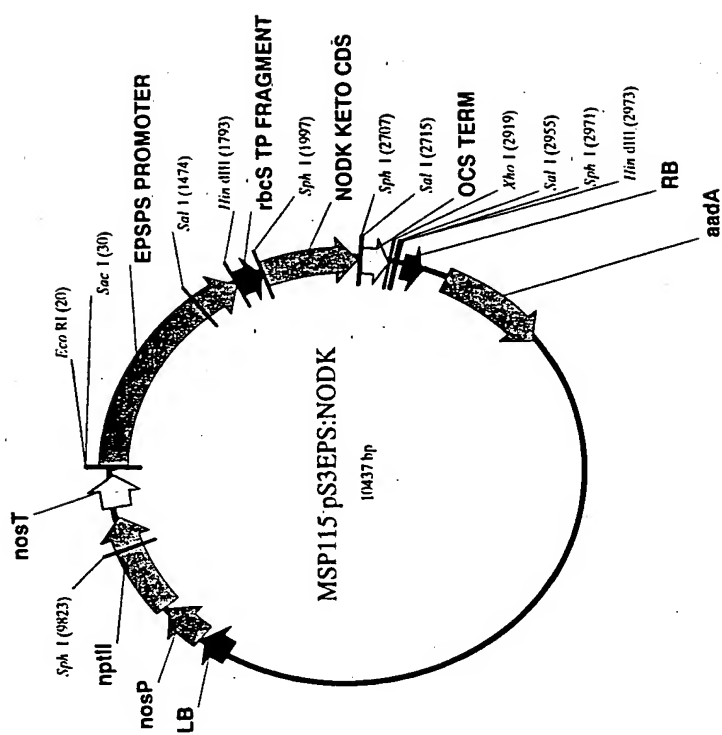


Figure 33: pSUN5 construct for overexpressing the β -C-4-oxygenase protein from *Nodularia spumigena* NSOR10 with rbcS transit peptide from pea under the control of the EPSPS promoter

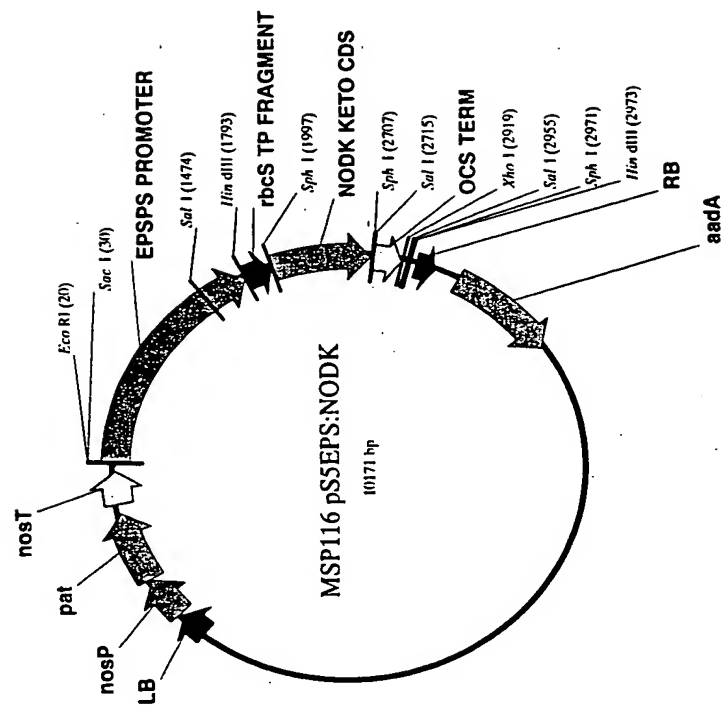


Figure 34: pSUN5 construct for overexpressing the β -C-4-oxygenase protein from *Nodularia spumigena* NSOR10 and downregulating the endogenous *Tagetes epsilon-cyclase* in *Tagetes erecta*

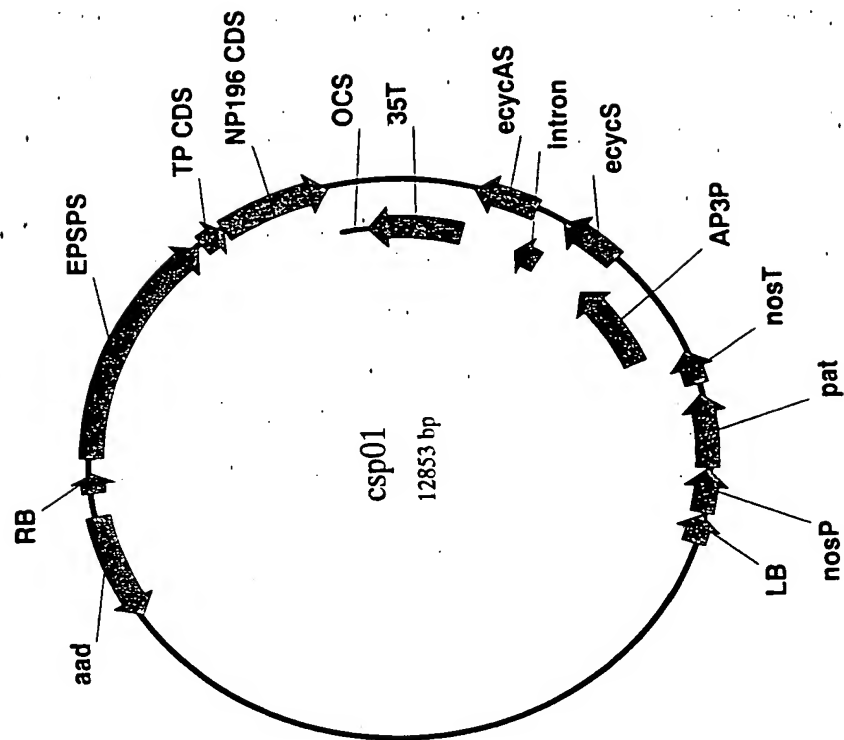
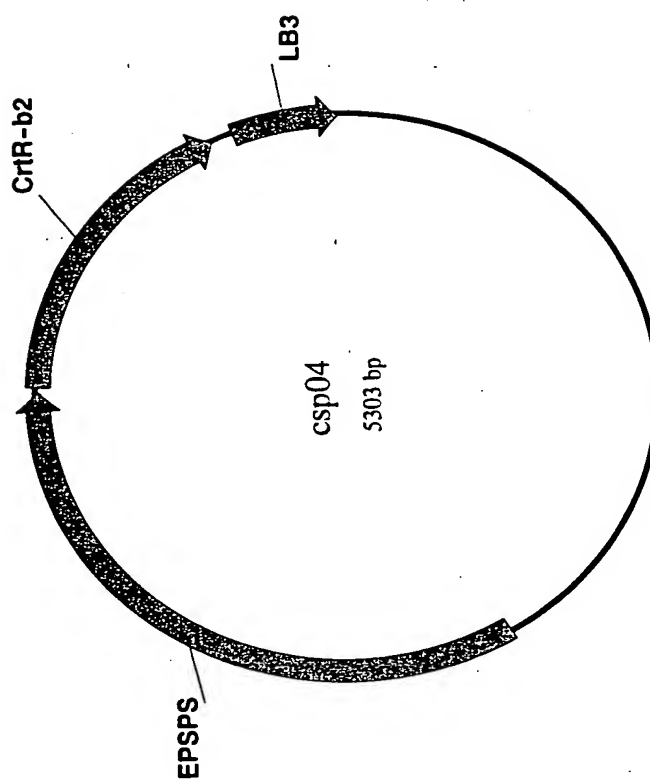


Figure 35: Expression cassette for overexpressing the β -hydroxylase from tomato under the control of the EPSPS promoter



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Figure 36: Expression cassette for downregulating the endogenous β -hydroxylase from *Tagetes* under the control of the EPSPS promoter

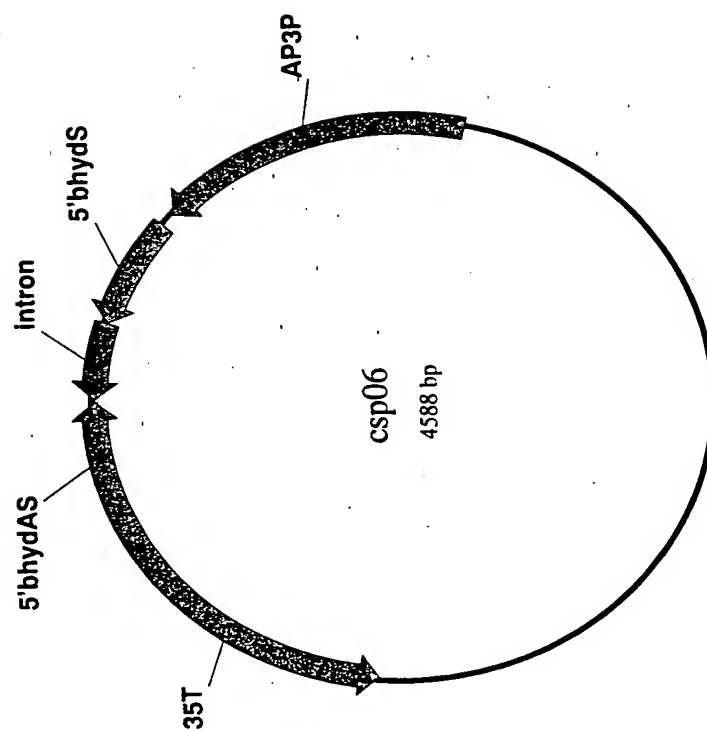


Figure 37: pSUN5 construct for downregulating the endogenous *Tagetes epsilon-cyclase* and overexpressing the NP196 ketolase and the tomato β -hydrolase

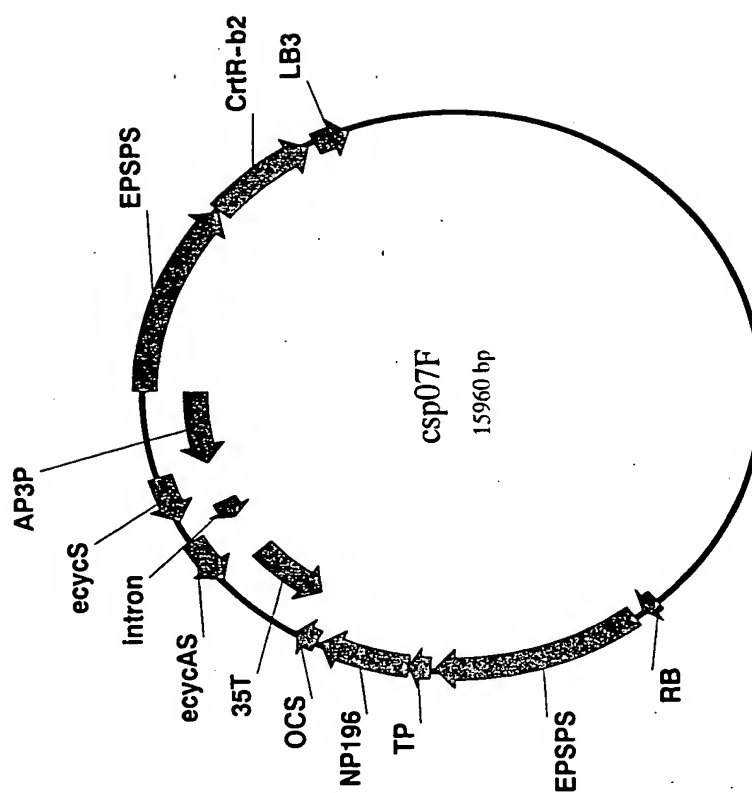


Figure 38: pSUN5 construct for downregulating the endogenous Tagetes epsilon-cyclase, overexpressing the endogenous Tagetes β -hydroxylase and overexpressing the NP196 ketolase and the tomato β -hydroxylase

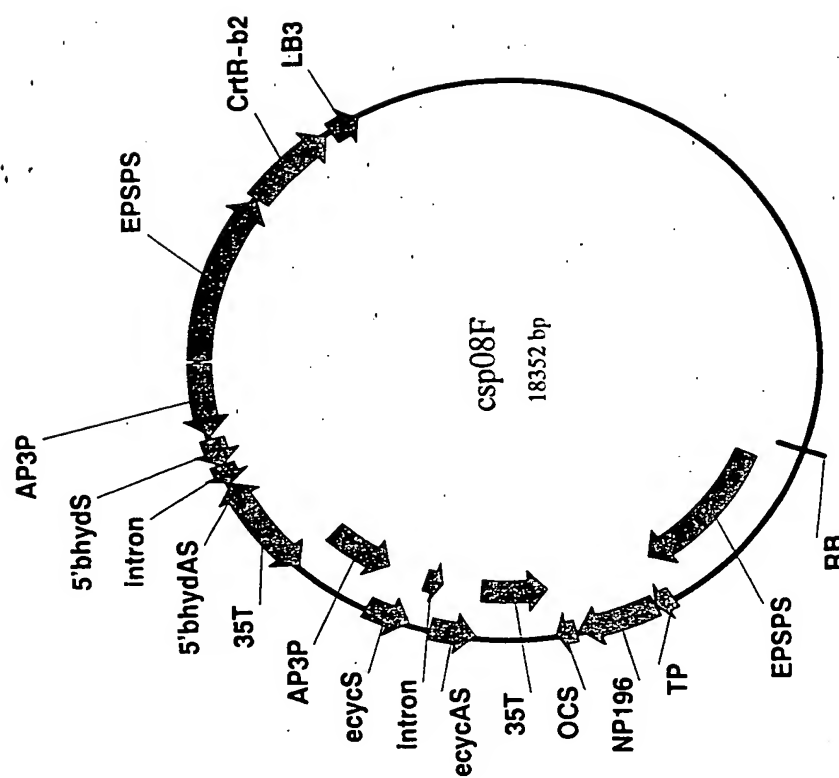
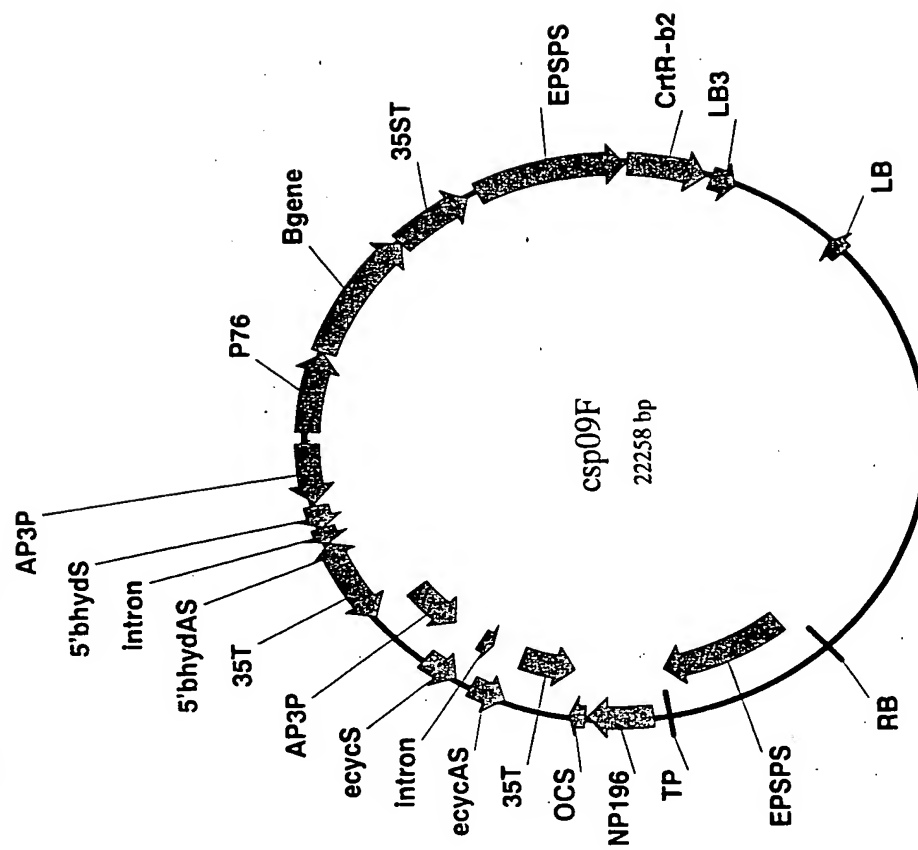
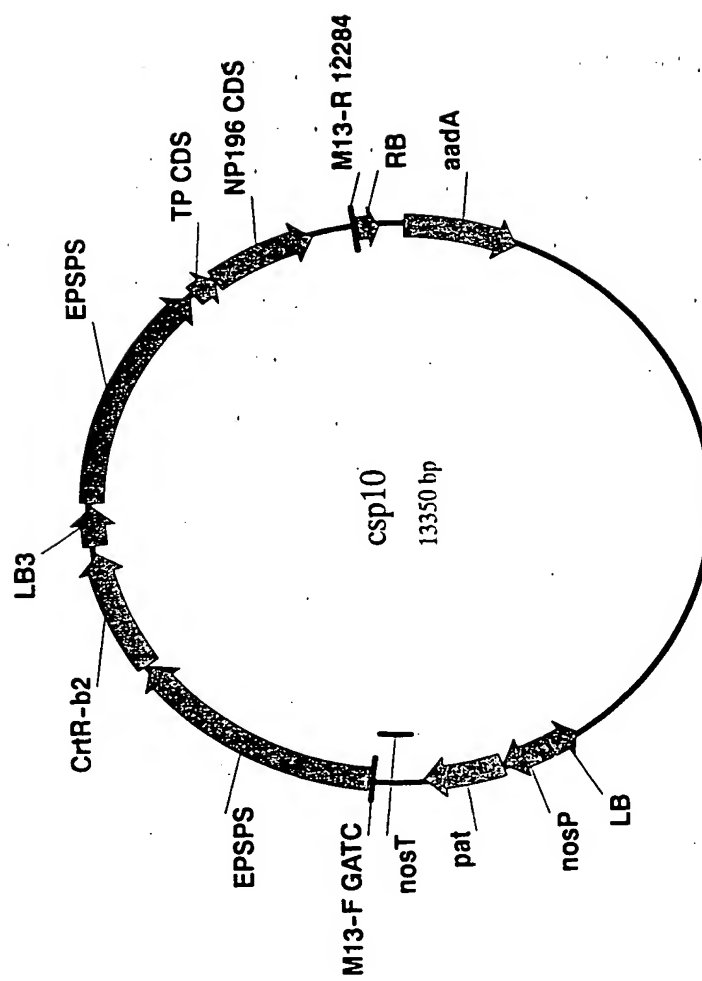


Figure 39: pSUN5 construct for downregulating the endogenous *Tagetes* epsilon-cyclase, downregulating the endogenous *Tagetes* β -hydroxylase and overexpressing the NP196 ketolase and the tomato β -hydroxylase and the B gene from tomato



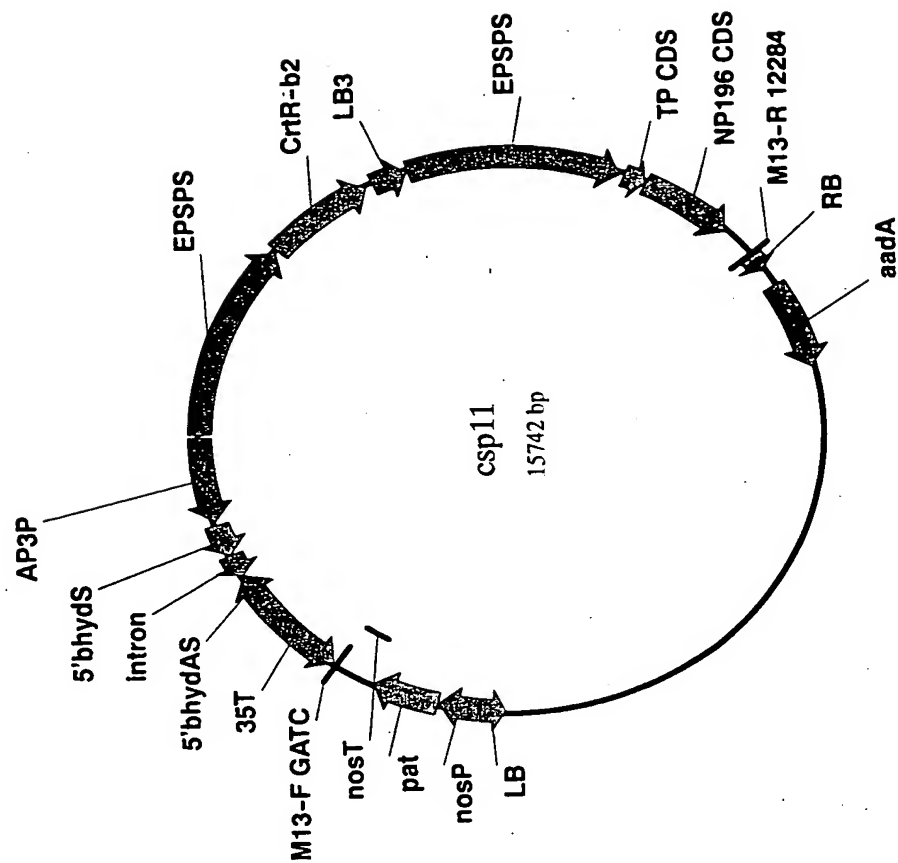
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Figure 40: pSUN5 construct for overexpressing the NP196 ketolase and the tomato β -hydroxylase



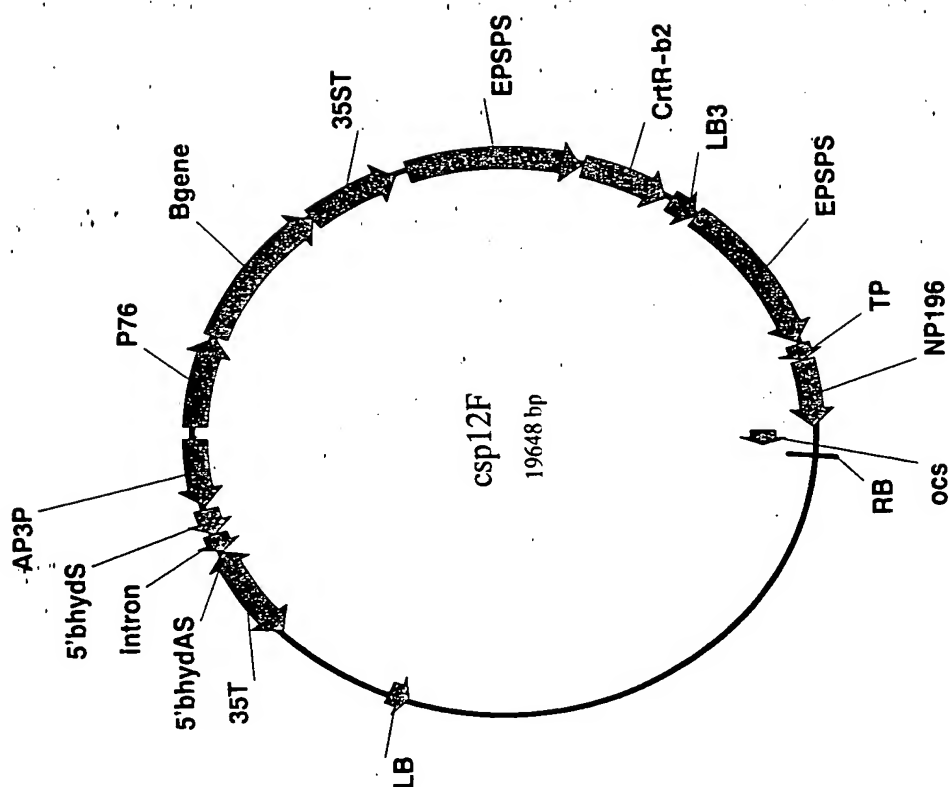
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Figure 41: pSUN5 construct for downregulating the endogenous Tagetes β -hydroxylase and downregulating the NP196 ketolase and the tomato β -hydroxylase



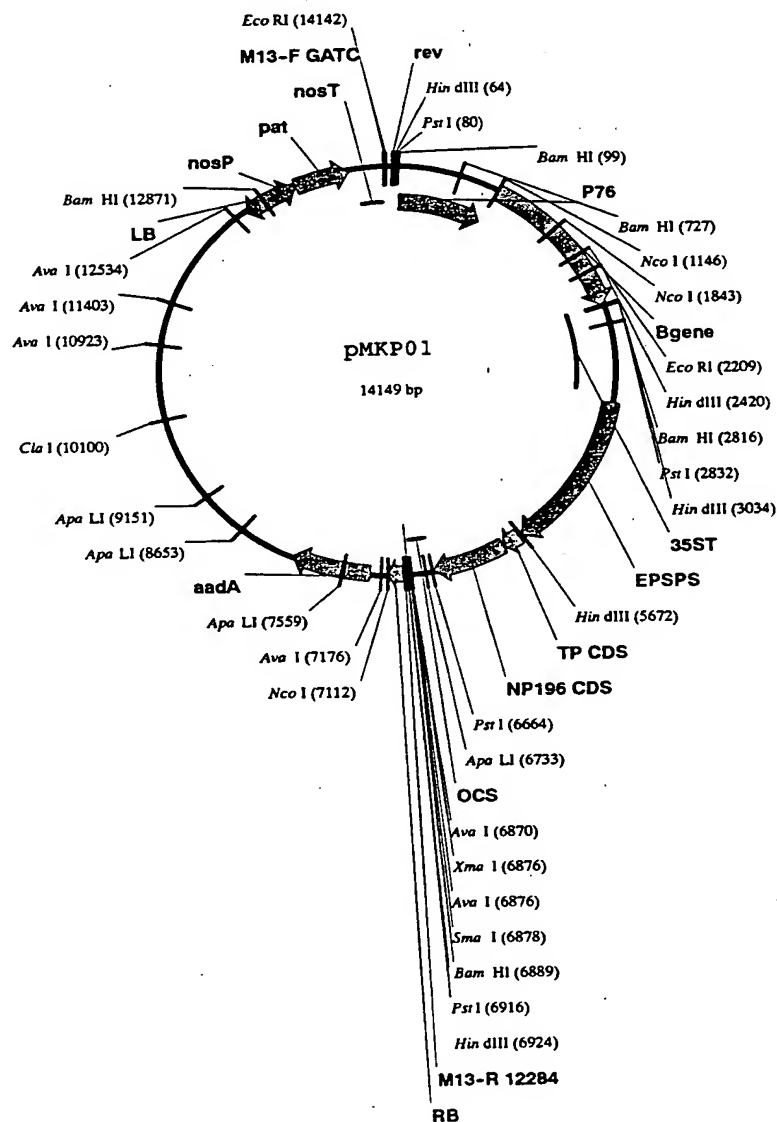
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Figure 42: pSUN5 construct for downregulating the endogenous Tagetes β -hydroxylase and for overexpressing the NP196 ketolase, the B gene and the tomato β -hydroxylase



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Figure 43: Expression vector for the flower-specific expression of the chromoplast-specific lycopene beta-cyclase from *Lycopersicon esculentum* under the control of the promoter P76 and for the flower-specific expression of the ketolase NP196 from *Nostoc punctiforme* ATCC 29133 under the control of the EPSPS promoter



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Figure 44: Expression vector for the flower-specific expression of the chromoplast-specific lycopene beta-cyclase from *Lycopersicon esculentum* under the control of the promoter P76, for the flower-specific expression of the ketolase NP196 from *Nostoc punctiforme* ATCC 29133 under the control of the EPSPS promoter and for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter

